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Short communication

First Record of *Callochiton foveolatus* (Polyplacophora: Callochitonidae) from Korea

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ABSTRACT

The genus *Callochiton* Gray, 1847 is small to medium sized chiton (up to 55×36 mm in size) and includes approximately 30 species worldwide, most of which are found on hard substrata in subtidal zone. To date, only three species of *Callochiton* Gray, 1847 have been reported in Japan. In this study, we found *C. foveolatus* (Is. Taki, 1938) as first record of the family Callochitonidae Plate, 1901 in Korea and its morphological features were described and compared with other northwestern Pacific species. In addition, the partial fragment of mitochondrial DNA cytochrome c oxidase subunit I sequences of *C. foveolatus* was determined as DNA barcoding record and compared with other congeneric species.

Keywords: *Callochiton*, Callochitonidae, radula, mtDNA *cox1*, Korea

INTRODUCTION

Callochitonidae Plate, 1901 is a medium-sized chiton family distinguished by having relatively broad girdle, smooth valves with well-developed insertion plates bearing many marginal slits on head and tail valves, respectively (Hayward and Ryland, 2017). This family includes four genera: *Callochiton* Gray, 1847, *Eudoxochiton* Shuttleworth, 1853, *Quaestiplax* Iredale & Hull, 1929, and *Vermichiton* Kaas, 1991. Approximately 30 species constitute the genus *Callochiton* (Kaas and Van Belle, 1985, Sirenko, 2006) and are distributed from Antarctica to the equator, of which only three species *C. foveolatus* (Is. Taki, 1938), *C. septemcostatus* Bergenhayn, 1933, and *C. multidentatus* (Carpenter in Pilsbry, 1892) were reported in northwest Pacific, Japan (Kaas and Van Belle, 1985).

We described morphological features of *C. foveolatus* (Is. Taki, 1938) with scanning electron microscope (SEM) photos of radula and valves, and mtDNA cytochrome c oxidase subunit I (*cox1*) sequence was determined, as its DNA barcoding record.

The specimens were collected from rocky shore in southern sea in Korea and fixed in 95% ethanol solution. We identified the specimens based on shell characters of head, tail, and intermediate valves using a stereoscopic microscope (Leica M205C, Wetzlar, Germany). For the examination of detailed morphological characters as valves, girdle, and radula using a SEM, one specimen was dissected and boiled at 50°C in 10% KOH solution for 2 to 5 min, then washed with distilled water. After washing, the remaining tissue was removed using Ultrasonic cleaner (Shinhan 200H 3L, Shinhan-Sonic, Korea). The valves, girdle and radula were coated with gold-palladium ion after drying. Ultramicroscopic morphological features of the radula and shell surface were observed using SEM (Ultra Plus, Zeiss, Germany).

For the mtDNA *cox1* barcoding of the specimens, genomic DNA was extracted from mantle tissue using the E.Z.N.A. Mollusc DNA Kit (OMEGA Bio-tek, Norcross, GA, USA). To amplify *cox1* partial gene fragment of mitochondrial DNA, polymerase chain reaction (PCR) was performed with universal primers (LCO1490, HCO2198) (Folmer et al., 1994) using TaKaRa Ex Taq (Takara Bio, Shiga, Japan); 37.25 μL of distilled water, 5 μL of 10× Ex Taq buffer, 4 μL of dNTP Mixture (2.5 mM each), 1 μL of each universal primers (LCO1490, HCO2198), 0.25 μL of TaKaRa Ex Taq and 1.5 μL of genomic DNA were mixed to make a total volume 50 μL. PCR amplification was performed as follows:

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initial denaturation at 95°C for 1 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 46°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72 °C for 10 min. The amplified PCR product was purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Sequencing reaction was performed by ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) in both directions. The mitochondrial *cox1* sequences *C. foveolatus* was deposited in GenBank (accession no. MG372123, MG372124). The mtDNA *cox1* sequences for three other *Callochiton* species available on GenBank were multiple-aligned using Clustal W (Thompson et al., 1994). Phylogenetic tree was reconstructed in MEGA 7 software (Kumar et al., 2016) using maximum likelihood method with 1,000 replications of bootstrap and HKY model (Hasegawa et al., 1985). Genetic distances of mtDNA *cox1* sequences were calculated using Tamura-Nei model (Tamura and Nei, 1993). The specimens of used to this study were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK) in Seoul, Korea (MMRBK no. 00005646, 0000 5647) and the National Institute of Biological Resources (NIBR) in Incheon, Korea (NIBR no. NIBRIV0000754909).

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Polyplacophora Gray, 1821
Order Chitonida Thiele, 1909
1*Family Callochitonidae Plate, 1901
2*Genus *Callochiton* Gray, 1847
Type species. *Chiton laevis* Montagu, 1803.

Diagnosis. Body small to medium size, head and tail valves with many slits, intermediate valves with 1–4 slits, perinotum covered with imbricated and inwardly directed spicules, reduced major uncinus tooth, reduced acrosome, long nuclear filament and symmetrical mitochondria of spermatozoid (Russell-Pinto et al., 1984; Kaas and Van Belle, 1985; Hodgson et al., 1988; Saito, 2004; Buckland-Nicks, 2008).

3****Callochiton foveolatus* (Is. Taki, 1938) (Figs. 1, 2)**

Spongioradsia foveolata Is. Taki, 1938: 344–347, Pl. 14, fig. 4, Pl. 20, fig. 1–10, Pl. 21, fig. 1; Is. Taki, 1962: 40; Iw. Taki, 1964: 409; Ferreira, 1982: 132; Kaas and Van Belle, 1985: 30, fig. 10.

Material examined. Korea: 1 individual: Gyeonsangnamdo, Irun-myeon, Gujora-ri, Gujora beach, 6 Jun 2016; 2 individuals, Jeollanam-do: Jindo-gun, Gogun-myeon, Geumgye-ri, Gagye seawall, 6 Jul 2016.

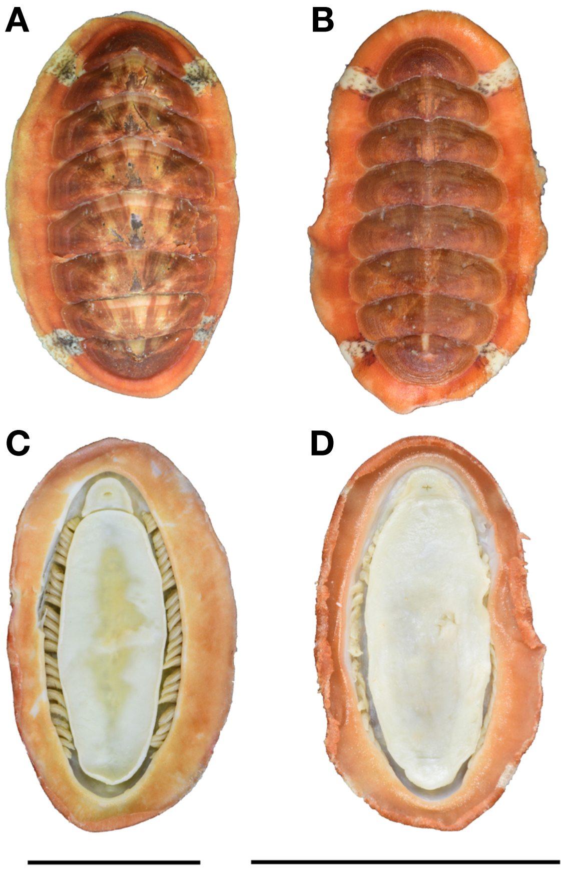

Fig. 1. *Callochiton foveolatus* (Is. Taki). A, Adult specimen, dorsal view; B, Juvenile specimen, dorsal view; C, Adult specimen, ventral view; D, Juvenile specimen, ventral view. Scale bars = 10 mm.

Description. Body oval, medium size (length 13–23.5 mm, width 8–12 mm), valves red colored (Fig. 1A, B). Gills adanal arrangement, holobranchial (Fig. 1C, D). Girdle covered with inwardly directed spicules. Pale red color with four narrow radial white bands. Bands located on the outer sides of the girdle between head valve and 2nd valve, and between 7th valve and tail valve.

Head valve red colored, semicircle; anterior margin rounded; posterior margin widely V-shaped with growth lines, small granules, and black shell eyes; no black shell eyes on posterior margin (Fig. 2A). Articulamentum pinkish white, quite thick; insertion plate irregular rectangle shape with 15 slits (Fig. 2B).

Intermediate valves with irregular longitudinal red lines on a yellowish beige background on central area (Fig. 2C),

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Korean name: 1*사방흰띠군부과(신칭), 2*사방흰띠군부속(신칭), 3*벌집사방흰띠군부(신칭)

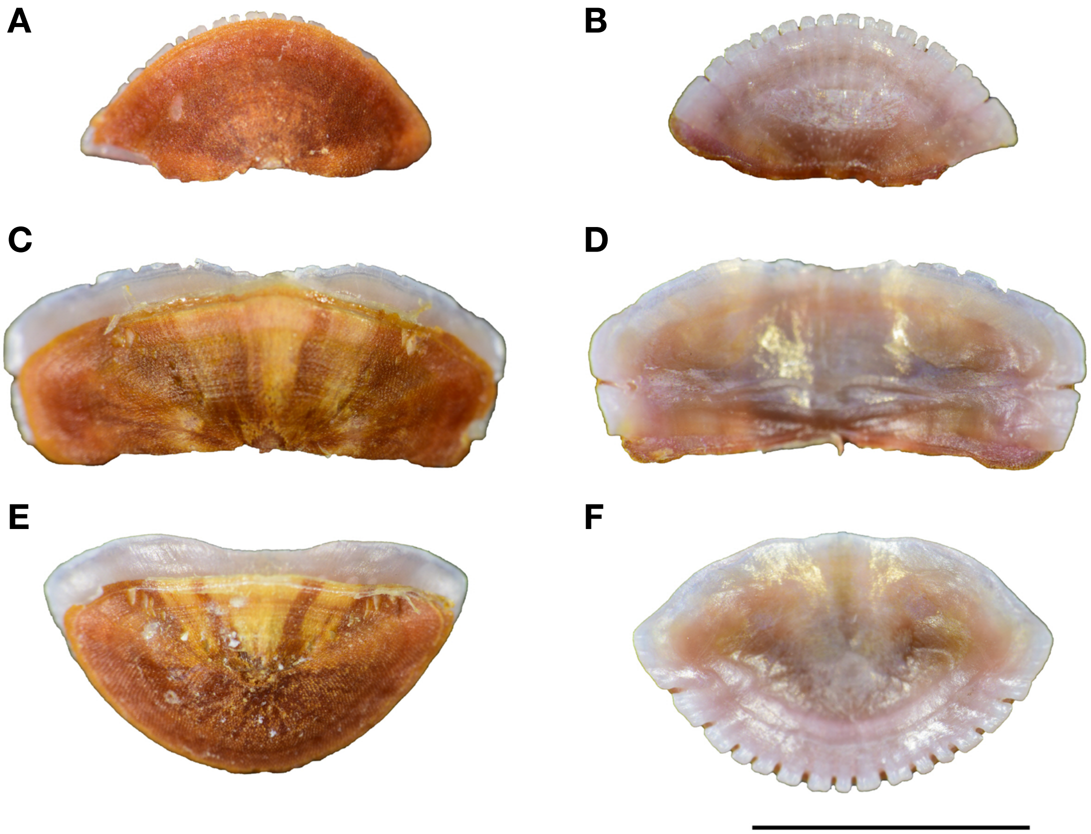

Fig. 2. *Callochiton foveolatus* (Is. Taki). A, Head valve, dorsal view; B, Head valve, ventral view; C, 7th valve, dorsal view; D, 7th valve, ventral view; E, Tail valve, dorsal view; F, Tail valve, ventral view. Scale bar = 5 mm.

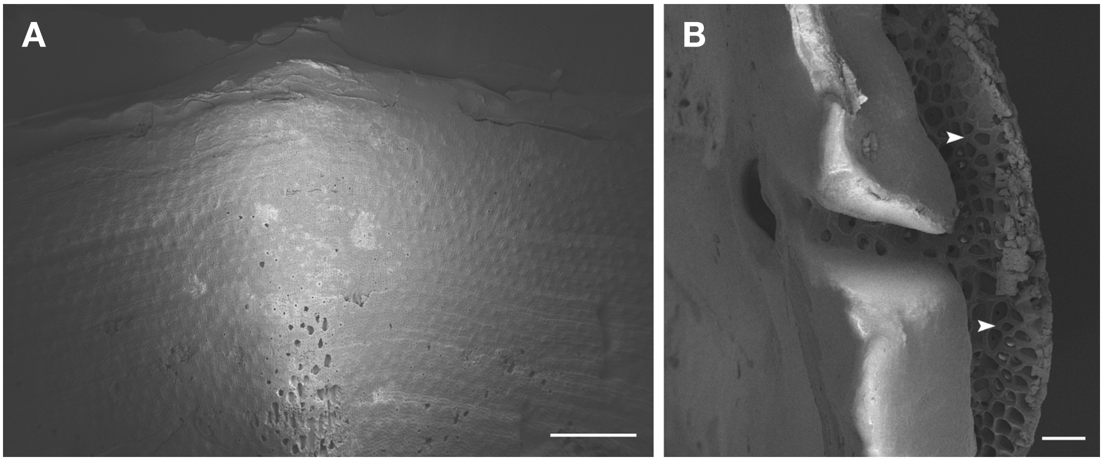

Fig. 3. *Callochiton foveolatus* (Is. Taki), microstructure of 7th valve. A, Surface on central area; B, Lateral view of side margin with eaves indicated white arrows. Scale bars: A = 500 μm, B = 100 μm.

short and broadly rectangular shaped, anterior margin of intermediate valves evenly convex, side margins straight (Fig. 1A) [but in juvenile specimen side margins rounded (Fig. 1B)], posterior margin somewhat arched with small rounded central apex; jugal and pleural area not distinguishable; central area smooth to the naked eye, but with very small pustules visible in SEM images (Fig. 3A); lateral area very weakly raised; black shell eyes present except for posterior margin; many spongy eaves (Fig. 3B); developed callus in the center of ventral area, anterior margin of articulamentum concave, 2 slits on the both sides (Fig. 2D).

Tail valve the same color as the central area (red or yellowish beige) on antemucronal area (Fig. 2E), semicircular, mucro central, antemucronal area with the same pattern as the central area of intermediate valves, postmucronal area with the same pattern as the head valve, insertion plate with 14 slits (Fig. 2F).

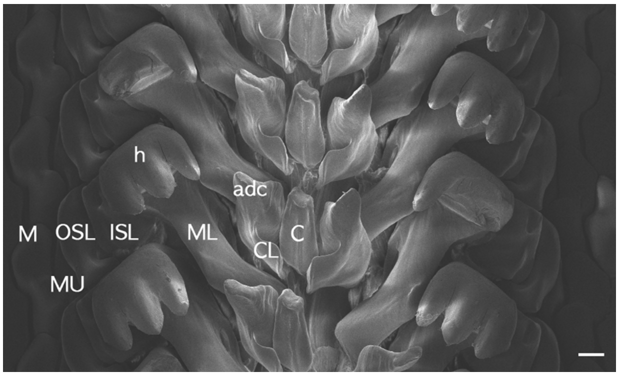

Fig. 4. *Callochiton foveolatus* (Is. Taki), radula, scanning electron microscope photograph. adc, antero-dorsal corner of centro-lateral tooth; C, central tooth; CL, centro-lateral tooth; h, head of major lateral tooth; ISL, inner small lateral tooth; M, marginal tooth; ML, major lateral tooth; MU, major uncinus tooth; OSL, outer small lateral tooth. Scale bar=20 μm.

Radula (Fig. 4) rows asymmetric. Central tooth oblong, narrow and a little dented near the anterior end. Centro-lateral tooth thick, anterior edge meandering, antero-dorsal corner protruding. Major lateral tooth with curved shaft, tridentate head, and three cusps of almost identical length; median cusp the thickest. Inner small lateral tooth squared on upper edge, grooved on lower edge. Outer small lateral tooth with rounded edge. Major uncinal tooth reduced. Marginal tooth flat (Fig. 4).

Habitat. Rocky shore at intertidal and subtidal zone.

Distribution. Korea and Japan.

Remarks. In the original description *C. foveolatus*, the slit formula was described as 15/2/13 (Is. Taki, 1938), but it differs slightly from the specimens collected from Korea (slit formula: 15/1/14) (Fig. 2B, D, F). We consider this difference is due to individual variation with different geographic origins, because in case of some other *Callochiton* species, individual variation in slit formula is also reported; *C. septemvalvis* was described as 16-20/2-3/14-16, or in some case, original description was solely based on morphological observation from a single specimen (Kaas and Van Belle, 1985).

Three *Callochiton* species have been reported thus far in northwest Pacific: *C. foveolatus*, *C. septemcostatus*, *C. multidentatus*. *Callochiton septemcostatus* is easily distinguished from the other two species by having the chalky white valves color, longitudinal ribs on central area of intermediated valves, quite raised lateral area with fewer shell

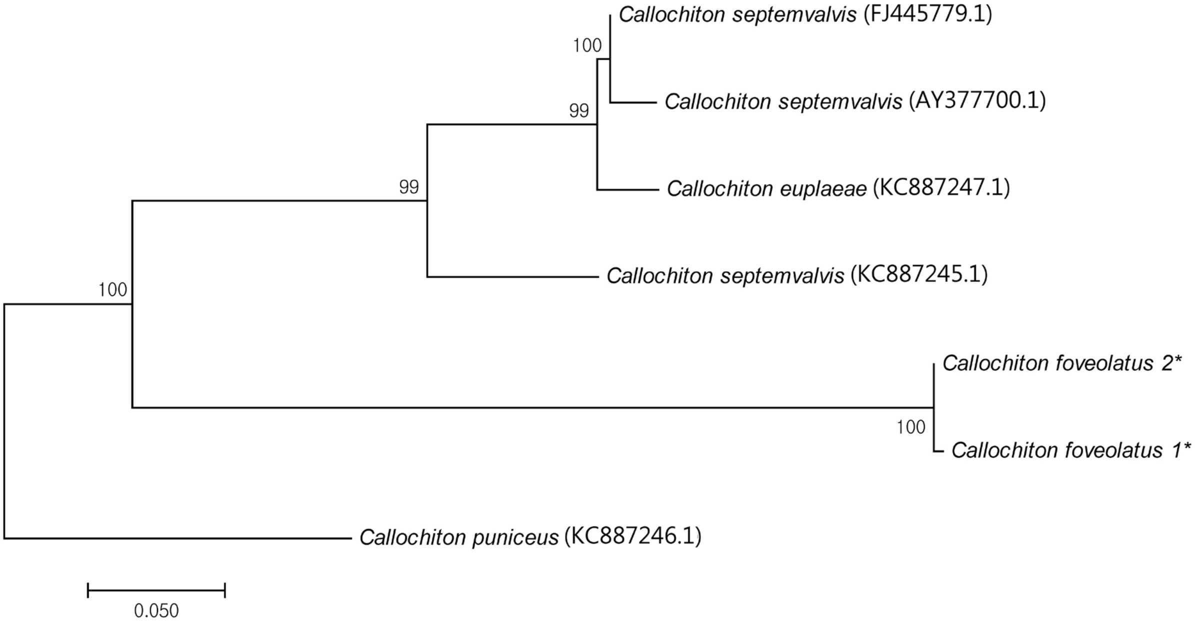
Callochiton septemvalvis (FJ445779.1)
100
Callochiton septemvalvis (AY377700.1)
99
Callochiton euplaeae (KC887247.1)
99
Callochiton septemvalvis (KC887245.1)
100
Callochiton foveolatus 2*
100
Callochiton foveolatus 1*
Callochiton puniceus (KC887246.1)
0.050

Fig. 5. Phylogenetic tree for mtDNA *cox1* sequence using maximum likelihood method. GenBank accession numbers were shown in brackets after species name. Asterisks (*) after species name indicate *cox1* sequences obtained in this study.

eyes. *Callochiton multidentatus* is similar to *C. foveolatus* in overall characteristics, but the former has oval shape granules on central area of intermediate valves and yellowish grey shell eyes (Kaas and Van Belle, 1985).

Callochiton foveolatus is similar to *C. septemvalvis* in many characters of valve and its coloration, but the latter is found in Europe including Norway, Denmark, United Kingdom, Ireland, France, Spain, and Portugal (Kaas and Van Belle, 1985). Despite their morphological resemblance, genetic distance between these species in mtDNA *cox1* is very substantial, ranging from 27.3% to 30.2%. Phylogenetic tree reconstructed using *C. puniceus* as an outgroup shows that *Callochiton* species are divided into two groups, each representing two geographical species groups: European group [*C. septemvalvis* and *C. euplaeae* (an invalid name as it is believed to be a junior synonym of *C. septemvalvis*; Dell'Angelo and Smriglio, 2001)], and Northwestern Pacific group (*C. foveolatus*) (Fig. 5). In this study, the northwestern Pacific group is represented by only a single species, *C. foveolatus* from Korea, and molecular analysis based on further taxon sampling of *Callochiton* species including northwestern Pacific species is required to understand phylogenetic relationships among the genus *Callochiton*.

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